

SICKLE CELL ANEMIA

- A PROFILE

- RAMESH RAO.

Uvekananda Girijana Kalyana Kendra, B . R . Hills



PREFACE

" IN THE LONG RUN, IF YOU
CANNOT TELL EVERYBODY WHAT
YOU ARE DOING, YOUR DOING
HAS BEEN WORTHLESS. "

In the following pages is a compilation of a few facts about Sickle Cell Anaemia.

An effort has been made to include as faithfully as possible, the 'happenings' in Vivekananda Girijana Kalyana Kendra with regard to Sickle Cell Anaemia.

Many facts have been left out, either due to lack of space or due to lack of understanding !

No amount of words can express my thanks to Dr. Sudarshan for giving me this opportunity to write, and also to the inspiration he gave me, just as he gave it to many young doctors. Let me not forget to thank Dr. Sudeep Nair for helping me in this compilation, Mr. Jayashekhar Murthy for the tabulating statistical data, Mr. L. Narendra - B.E (Com. Sc.,) for the beautiful graphs, and all others who have been directly or indirectly involved in the Sickle Cell Research here.

- DR. B. S. RAMESH RAO

IN THE FOGHORN I GO
CANNOT TELL WHERE I AM
YOU ARE DREAMING OVER DOG
BECOME MORTALITY

(the old woods about wet in noiseless air were gathered cold at
dawn)

and suddenly as lightning at edge noon and winter, an
accident of dragon in the earthy smoke which emanated from "Fog" and
was carried by the wind to the sea.

that of one to sleep he said of the people who had died over there
I gathered to

in private yet, perforce, 10 of them in houses see above to drown off
heat and sleep as sufficient aid of cold and warmth of atmosphere and
the wind as comforter am the best guides you can in sleep as
you will understand. This condition stiff in emulsion you will find
itself, and 2.H. 11. after which an auto-traveler will travel fast and
to visitors need over the winter the best before I finished and not
over houses left in before visitation

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КОМПОЗИЦИЯ — АЛЛЕГРА ДАС САМОЕ

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свободы *

имеет право на употребление
своей власти *

СОСТАВЛЕНИЕ САМЫХ ПРОСТОРНЫХ

и ярких *

аллегорий и сюжетов из истории
один разбрасывает в альбомах

один раз расставляет в залах
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SICKLE CELL ANAEMIA - AN INTRODUCTION.

HISTORY :

Sickle Cell Anaemia is a genetic disease which results in the production of abnormal haemoglobin.

The disease has existed since many thousands of years, but a formal report of it was made only in 1910 by Herrick, a Chicago Physician, when he wrote about "an intelligent Negro" who had "unusual blood findings" and wondered whether the blood picture represented "merely a freakish poikilocytosis" !

Later on, there were many reports on similar cases, and it was Mason, who in 1922, coined the term 'Sickle Cell Anemia'. In 1949, in their paper 'Sickle Cell Anemia' - a molecular disease', Pauling et al, elucidated the molecular basis of the sickling phenomenon.

Allison, in 1954 proposed that Sickle Cell trait was protective against falciparum malaria. Through this arose the concept of balanced polymorphism in Sickle Cell disease.

Ingram, in 1956 showed that the change was due to the substitution of Valine, to the normal Glutamic acid, in position six of the beta chain (yielding a loss of two negative charges per Hb molecule).

PHYSIOLOGY

Haemoglobin is the respiratory pigment of the Erythrocytes. It is ovoid in shape and consists of two parts - Haem and Globin. Haem consists of an Iron core and four porphyrin groups surrounding it. Oxygen reversibly binds to the Iron moiety.

The globin part consists of four Globin chains. In the normal adult there are 2 alpha and 2 beta chains. A Haem group is attached to each globin chain. Thus, each haemoglobin molecule consists of 4 Haem groups and 4 Globin chains, closely associated with each other.

Even in the normal human different types of Haemoglobin are synthesised during different periods of life. The type of the Haemoglobin depends on the combination of the Globin chains.

The different types of Globin chains and Haemoglobins synthesised by normal human beings are illustrated in FIG's I.1, I.2, I.3.

FIG. I.1

TYPES OF HUMAN GLOBIN CHAINS

1. ALPHA α
2. BETA β
3. GAMMA γ
4. DELTA δ
5. EPSILON ϵ
6. ZETA ζ

FIG. I.2

PERIOD OF LIFE	SITE OF SYNTHESIS	TYPES OF CHAINS SYNTHESIZED
EARLY EMBRYONIC	YOLK SAC	α, ϵ, ζ
FOETAL	LIVER ; SPLEEN	α, γ
POSTNATAL	BONE MARROW	α, β, δ

FIG. I.3

TYPES OF NORMAL HUMAN HAEMOGLOBINS :

1. Early Embryonic Life

- Hb Gower I - $\zeta_2 \epsilon_2$
- Hb Gower II - $\alpha_2 \epsilon_2$
- Hb Portland - $\zeta_2 \gamma_2$

2. FOETAL LIFE

- Hb F - $\alpha_2 \gamma_2$

3. POSTNATAL LIFE

- Hb A - $\alpha_2 \beta_2$

- Hb A₂ - $\alpha_2 \delta_2$

GENETICS :

The gene coding for the Alpha Globins is located in the short arm of chromosome 16 and those coding for the beta-globins is located in the short arm of chromosome 11.

In Sickle Cell Anaemia, there is a point mutation at the 6th codon for the beta chain from G A G to G U G resulting in the substitution of Valine to Glumatic acid at the 6th position of the beta chain ($\beta 6 : \text{Glu} \rightarrow \text{Val}$) This mutation is carried over as a recessive trait from one generation to the other.

Studies with Restriction Endonucleases (Enzymes which cleave DNA molecule at specific sites) have shown that the sickle cell mutation occurred independently, in different populations, several times, on chromosomes manifesting a variety of different haplotypes, probably some 3000 - 6000 generations ago (70,000 - 150,000 years ago) [Kurnit, Solomon & Bodmer -1979]

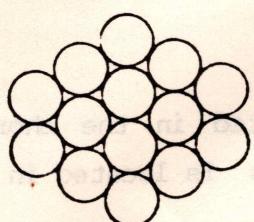
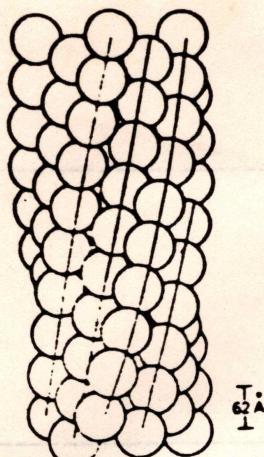
Regarding the migration of the Sickle Cell Gene to different geographic locations, Kirk says that - "...although HbS in East Africa seems to be derived from India via the Middle East, the West African HbS gene arose independently."

Central African populations derived its HbS gene from both sources.

PATOPHYSIOLOGY OF SICKLE CELL ANAEMIA

The deformity of the normally discoid and pliable Erythrocyte to the impossibly and rigid sickled cell is the key event for which we have to examine the pathophysiology.

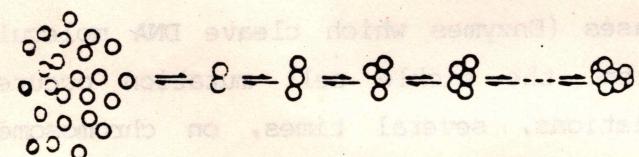
FIG. I. 4



The 14 Stranded Structure
of the deoxy-HbS polymer
as proposed by Dykes et al.

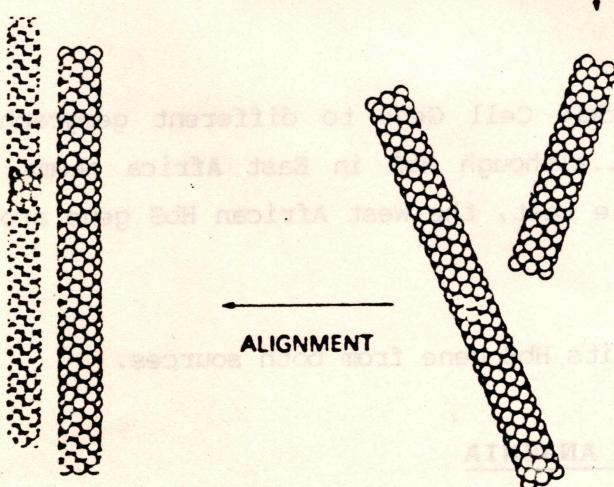
FIG. I. 5

NUCLEATION



Multi stage model of
polymer formation as
proposed by Hofrichter.
et al.

GROWTH



ALIGNMENT

PATENTED TECHNOLOGY OF SALKYE QCL AN

A 'Sickle RBC' is one that contains the 'Sickle Hb'. Once this sickle Haemoglobin is exposed to certain conditions in the Erythrocyte, it undergoes polymerization. This HbS polymer is described as a 14 standard structure, stacked in a gradual helix around a vertical axis. (FIG. I. 4)

According to Hofrichter, this polymer is formed gradually in stages, like nucleation, growth, alignment (FIG.I.5). The presence of the HbS polymer within the RBC severely reduces its pliability and turns it into a bizarre shaped rigid cell.

The normal RBC's manouvre through capillaries and venules much smaller than their diameter. However, when sickling occurs, the rigid RBC cause obstruction of the vessels at various levels, reducing the blood flow to the region and perpetuating the hypoxia which in itself is a potent stimulus for sickling.

There are many factors which promote the sickling of erythrocytes (invitro).

These are :

- Decreased oxygen tension
- Decreased PH
- Increased duration of conditions which may cause sickling
- Decreased temperatures
- Increased age of erythrocytes
- Increased concentration of HbS in erythrocytes

Sickle Cells tend to lose K^+ ions and accumulates Ca^{++} ions. This mechanism was proposed by Kirk as lethal to the malarial parasites infecting the RBC's.

CLINICAL FEATURES :

Sickle Cell disease was called a 'grand' disease due to its protean manifestations. The 'Sickle Cell crisis' is the hallmark of the disease, it can mimic many 'acute' conditions in our body. Eg : duodenal ulcer, acute appendicitis, etc.,

However, the clinical picture may vary, not only from patient to patient, but also from one part of the world to another !

Any precipitating factor may cause the erythrocytes to sickle, resulting in ischemia and infarction of the tissues. Moreover, the impliable RBC's are trapped in the splenic sinusoids - resulting in haemolytic anaemia. This causes splenomegaly. However, splenomegaly can be seen only in children and the spleen undergoes chronic infarction and fibrosis resulting in shrinkage, a process known as 'autosplenectomy'.

The patient may present any one of the following symptoms and signs:

- SYMPTOMS : 1. Painful crisis in - extremities ; abdomen ; chest-associated with passage of dark urine.
2. Symptoms of acute cholecystitis ; duodenal ulcer ; acute appendicitis.
3. Priapism - Haematuria.
4. Head-ache ; convulsion ; history of stroke ; history of visual field defect.
5. Episodes of acute bone pain and persistent joint pain.
6. Repeated Respiratory infection.

- SIGNS : 1. Pallor ; Jaundice.
2. Frontal Bossing.
3. Cutaneous ulcers (especially leg)
4. Splenomegaly ; hepatomegaly.
5. Evidence of neurological deficit or visual field defect.
6. Bony deformities of hands and feet ; limited mobility of joints ; evidence of osteomyelitis.

It can be said that the life expectancy of a Homozygous patient after 30 years is 30%. But, in the Soligas, as well as in other Tribes in South India, the disease runs a milder course. The subjects are clinically well, with fewer crises and a longer duration of life.

SICKLE CELL ANAEMIA - IN THE SOLIGA TRIBE .

THE SOLIGAS

The Soligas are forest dwelling aborigines of South India scattered along Biligiri Ranga Hills, Mahadeshwara Hills, forests of Bandipura, Hunsur, Kushalnagar and the Nilgiri Hills.

This tribe is a genetically distinct endogamous population with little or no admixture among other tribes or non-tribes. Investigations have suggested that the Soligas were in a long standing genetic isolation. In particular, no evidence of a genetic connection with Africa has been found.

The Soligas have a rich culture of their own and their isolation has helped them to preserve their uniqueness.

The Soliga lives an anxiety-free life, in harmony with the nature around them. His dress is simple and so is his food. The melodious and rhythmic 'Soliganudi' which he speaks, and the sweet songs he sings in praise of nature and his traditional Gods, is ever enchanting to listen.

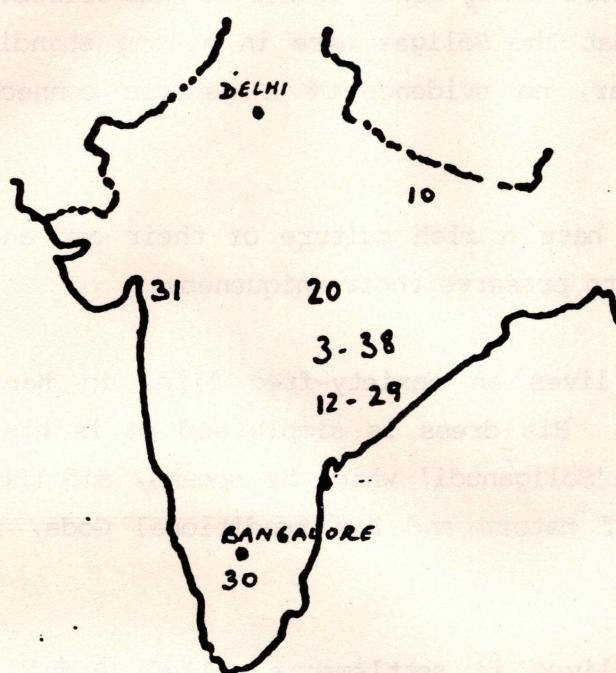
The Soliga lives in settlements called 'Podu'. Each Podu may have about 10-15 huts. These huts are small and beautiful; made of easily available materials like bamboo, straw and mud.

Ofcourse, today we see in many Podus, the brick and tile houses constructed for the Soligas by the Forest Department and other agencies.

BEAUTIFUL BILIGIRIRANGANA HILLS

Approximately 90 Km away from the city of Mysore, are the grand ranges of the Biligirirangana Hills, with a beauty that can bring any poet to ecstacy. The ranges, with their soft plateaus and peaks continue with the Western Ghats and other hills like the Nilgiri Hills and Maleya Mahadeshwara Hills.

FIG. II. 5



Distribution and prevalence of the Sickle Cell trait in India. (Figures given are percentages)

On one of its granite peaks is the temple of the Lord Bilingiri Rangaswamy, whom the Soligas call their 'Bhava' (Brother-in-law).

There are historical events attached to this place since ancient times, and one can go through the 'Sthala Purana' in a book published by the temple. The Vivekananda Girijana Kalyana Kendra is located nearby and bustles with activity from dawn to dusk.

SICKLE CELL ANAEMIA IN SOUTH INDIAN TRIBES AND OTHER PARTS OF INDIA.

Sickle Cell Anaemia in Indians was first discovered in an Indian patient living in South Africa by Burk and Bull in 1943. In 1952, Lehman and Cutbush discovered that Sickling occurred in high frequency among certain tribal groups in South India. Lehman, later found out that Sickle Cell Anaemia was present in many tribal groups in Central India and Gujarat.

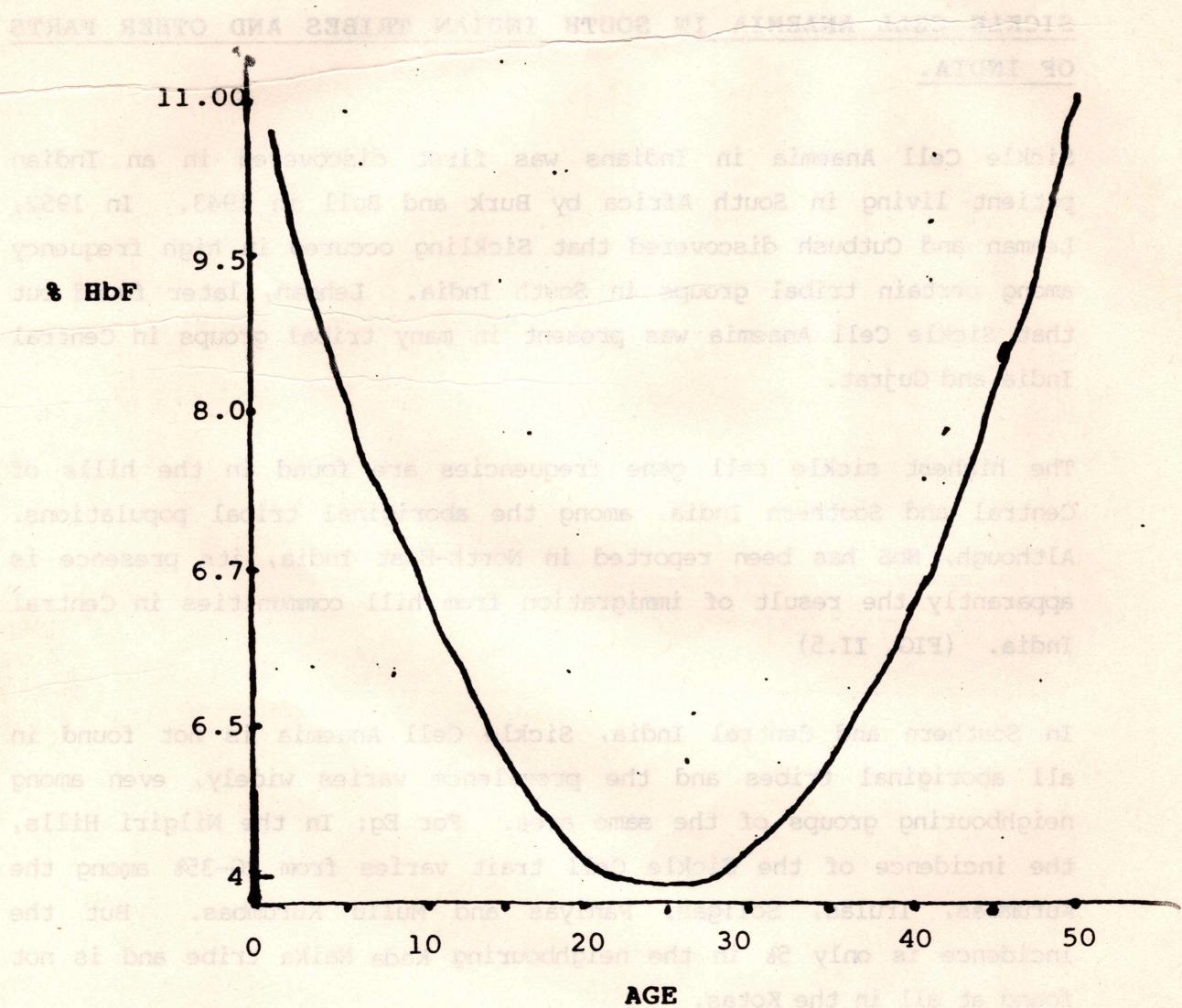
The highest sickle cell gene frequencies are found in the hills of Central and Southern India, among the aboriginal tribal populations. Although, HbS has been reported in North-East India, its presence is apparently the result of immigration from hill communities in Central India. (FIG. II.5)

In Southern and Central India, Sickle Cell Anaemia is not found in all aboriginal tribes and the prevalence varies widely, even among neighbouring groups of the same area. For Eg: In the Nilgiri Hills, the incidence of the Sickle Cell trait varies from 20-35% among the Kurumbas, Irulas, Soligas, Paniyas and Mullu Kurumbas. But the incidence is only 5% in the neighbouring Kada Naika tribe and is not found at all in the Kotas.

Similarly, the presence and prevalence of HbS in Central Indian tribal groups are not uniform, even in populations living in the same area. Interesting to note, is the presence of HbS in some non-tribal Hindu populations.

Prevalence of 9.6% of the Sickle Cell trait is observed among the Danukhs of Uttar Pradesh, 38.2% among the Mahars of Madhya Pradesh and Maharashtra, and 26.8% among the Sorathis of Gujarat.

FIG. II. 1



Curve obtained after studying the pattern of HbF in different ages in a Brazilian group of Homozygous patients. (Hutz, Salzano, & Adams. 1983)

The HbF levels were 11% before 5 years of age and dropped down to 4% by 20 years. Again the HbF levels increased from 30 years onwards. The higher HbF levels in patients above 30 years of age may explain their longevity. Compare with FIG. II. 3

The Soligas have an incidence of nearly 28% for the Sickle Cell trait (Dr. Sudarshan et al), and the incidence of Homozygous Sickle Cell disease is about 2%.

SPECIAL FEATURES OF THE DISEASE IN THIS POPULATION.

Though Sickle Cell Anaemia is commonly described as a 'Chronic Haemolytic Anaemia interspersed with painful crisis and a considerably shortened life span', the disease was found to be much benign in this population and also in the other tribal groups in various parts of India.

This benign form of the disease has also been noted in Saudi Arabia and Iran.

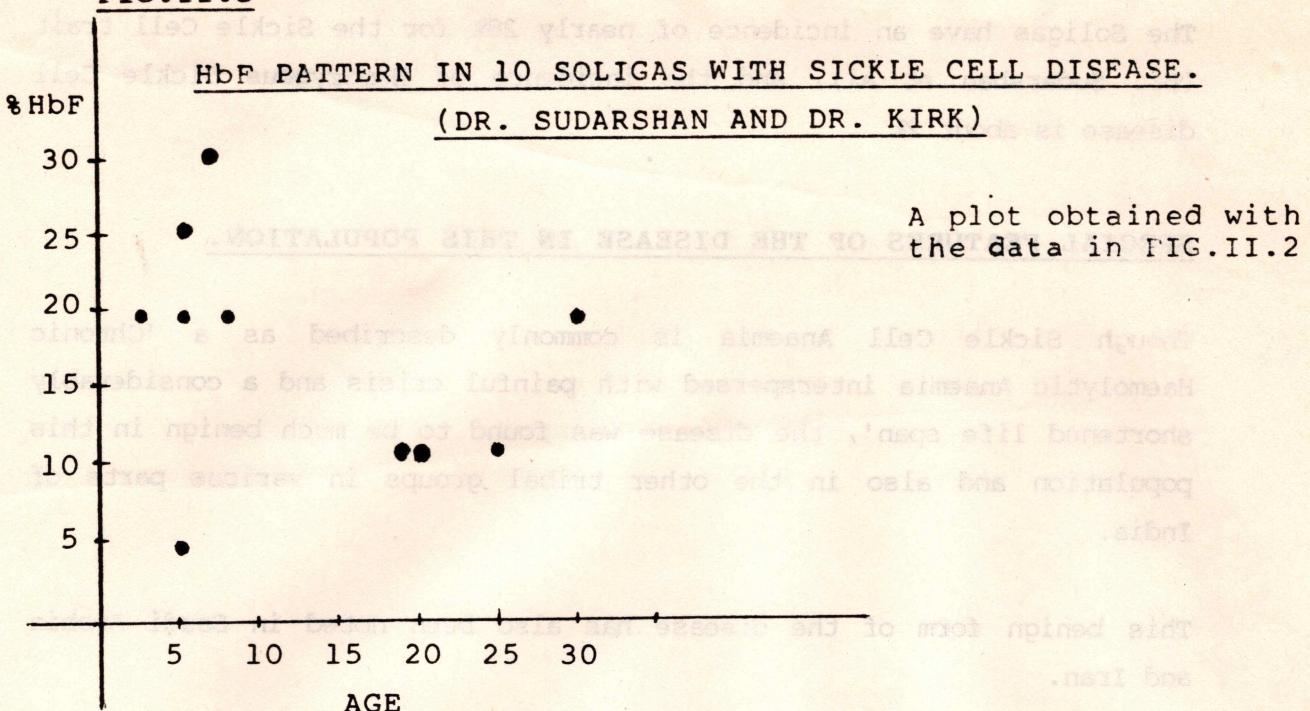
The most widely given explanation for the benign manifestation of the disease, is the elevated levels of haemoglobin in F in these patients. (FIG's II.1, II.4)

FIG. II.2.

SICKLE CELL DISEASE AND HbF LEVELS IN 10 SOLIGAS :

NO.	SEX	AGE	% HbF
1.	M	6 Yrs.	29
2.	F	5 Yrs.	24
3.	M	4 Yrs.	19
4.	M	6 Yrs.	19
5.	F	2½ Yrs.	19
6.	F	30 Yrs.	16
7.	F	25 Yrs.	10
8.	M	20 Yrs.	10
9.	M	20 Yrs.	10
10.	M	5 Yrs.	4
MEAN			16.0

FIG.II.3



HbF is composed of two alpha chains and two gamma chains. It is the predominant haemoglobin in foetal life. Synthesis takes place mainly in the liver and yolk sac.

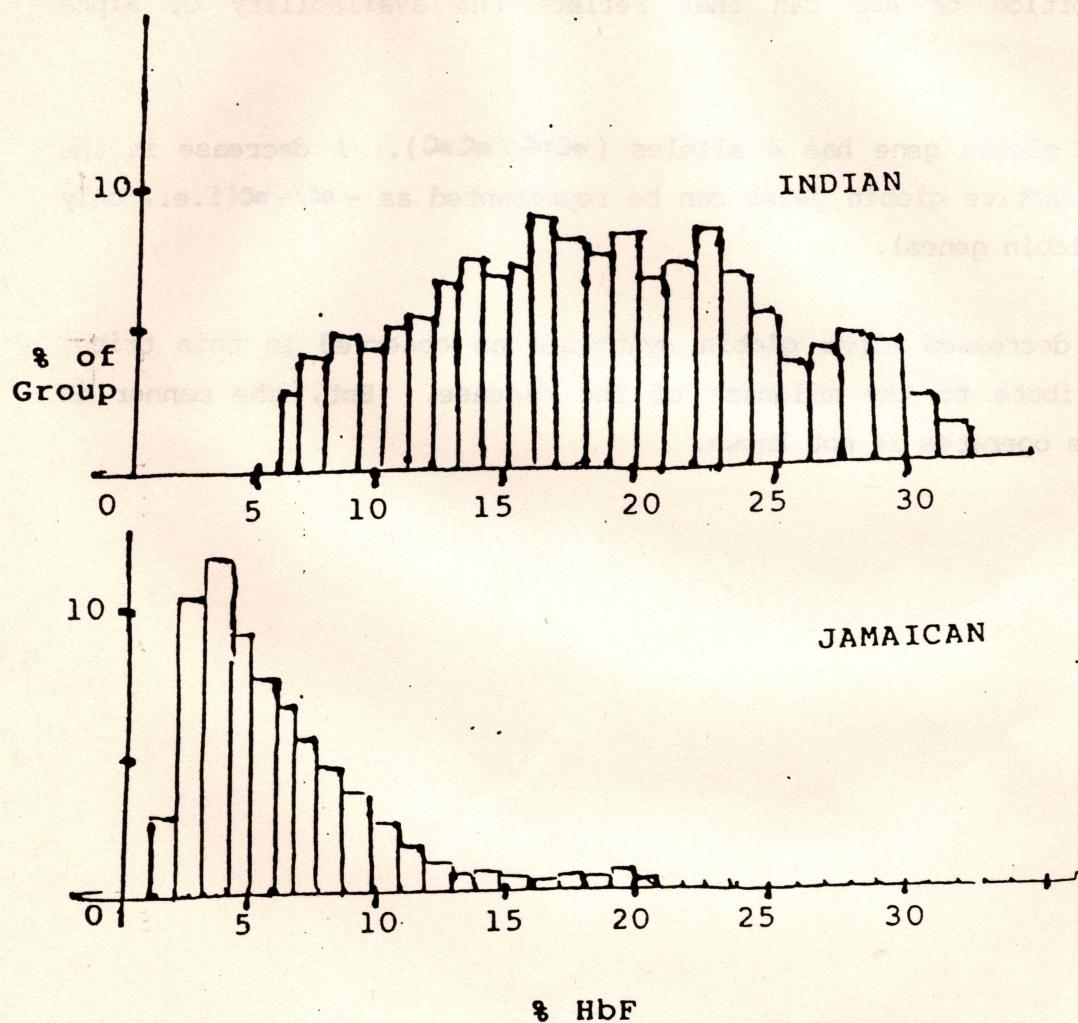
The genetic basis of the ability to produce high level of HbF is uncertain; but Kirk postulates in his study of the Saudi population, that "there may be an allele....which confers increased sensitivity to HbF production associated with increased Erythropoiesis."

How far this is true with the Soligas, is yet to be studied.
(FIG.II.2 & II.3)

Presence of HbF in sufficient concentrations allays the phenomenon of sickling in RBC's.

Another interesting feature observed by Brittenham was that the concentration of HbS in Heterozygotes is significantly less than 50%. (Normally, one would expect 50% HbS and 50% HbA). This was true of all the tribal populations he studied including the Soligas. This would mean that lesser amounts of HbS are available to participate with sickling process, i.e., RBC's will not sickle as readily.

FIG. II. 4



Comparison between the HbF levels of Indian patients and Jamaican patients (whose clinical severity is known to be more than Indian counterparts). The mean HbF value of 4.6% seen in the Jamaican group, was the lowest value seen in the Indian group. (Kar, Sathapathy, et al 1986)

Brittenham explained this phenomenon as due to a variation in the number of active alpha-globin genes.

Experimental evidence suggests that alpha chains have a greater affinity for beta-A chains than beta-S chains. (Thus, for example, if 15 α chains were to be presented to 10 β^A chains and 10 β^S chains, the outcome would be 10 HbA Molecule and 5 HbS Molecule). Conversely, the proportion of HbS can thus reflect the availability of alpha chains.

The alpha globin gene has 4 alleles ($\alpha\alpha\alpha\alpha/\alpha\alpha\alpha\alpha$). A decrease in the number of active globin genes can be represented as $-\alpha/-\alpha$ (i.e., only 2 alpha globin genes).

Thus the decreased alpha globin synthesis as observed in this tribe, may contribute to the mildness of the disease. But, the manner in which this operates is not known.

VIVEKANANDA GIRIJANA KALYANA KENDRA

AND SICKLE CELL RESEARCH

INTRODUCTION

Vivekanada Girijana Kalyana Kendra is a voluntary organisation founded in 1881, with the objective of serving the Soligas.

Starting from a scratch, Dr. Sudarshan has led the organisation to what it is today, with a wide range of activities, like, Health, Education, Vocational training, Adult education, Community organisation, Staff development programmes, etc.,

With the ideal of 'Service of God in man', V.G.K.K. has reached out its compassionate hand to thousands of tribals, in and around B.R.Hills, M.M.Hills, Yelandur, Kollegal and Chamarajanagar Taluks.

The status reached by the Soligas of today, in their overall development - breaking away from the shackles of their aboriginal past and standing face to face with the modern Indian, verily bears testimony of fructification of the years of toil by the dedicated workers here.

JAYAVIJAYAM TRIBAL HOSPITAL

The Medical wing of V.G.K.K. comprises of the Jayavijayam Tribal Hospital and the Jayavijayam Mobil Unit. The Hospital is well equipped with a ten bed ward, minor surgical facility, X-ray and screening unit, a well equipped laboratory and a fully equipped dental clinic.

In addition to carrying out various health and health related programmes such as immunizations, Health education, Health Worker Training, Traditional midwives training, Nutritional programmes, MCH programme, Herbal Medicine training etc., the medical wing has taken up some research programmes; prominent among which is the 'Sickle Cell Anaemia - screening and survey.'

The other ongoing studies are "Perineal tears in the Soliga Women" and "Blood Pressure - a survey and study in the Soligas."

Moreover, there are many doctors from India and abroad who have come here to do research and study in the Tribal population.

RESEARCH IN SICKLE CELL ANAEMIA

Ever since Herrick described the first case of Sickle Cell Anaemia in 1910, innumerable papers have poured in, year after year, on the various aspects of Sickle Cell Disease. Ironically, there is no cure as yet for the disease.

Ever since the inception of V.G.K.K, Dr. Sudarshan has put in pioneering efforts in screening the Soliga tribe for Sickle Cell Anaemia. The process still continues and nearly 2,000 tribals have been screened for the disease.

Dr. R. L. Kirk, former professor of Human Biology at the Australian National University, Canberra, is helping in this programme. Research is also being conducted to assess the clinical severity of the disease in this population. As already mentioned, the disease runs a comparatively benign course in this population, compared to West African and Black American subjects.

A group of ophthalmologists from Bangalore are conducting an ongoing study in the Soligas, with particular emphasis on ocular changes in Sickle Cell Anaemia.

Dr. Sudarshan and Dr. Kirk have studied the HbF pattern in a group of patients with Sickle Cell Disease.

RECORDS AND FILES

Family records are being maintained and classified according to the Podus. Each family is given a code number and the Sickle Cell Status marked for every member of the family.

In addition, separate cards are maintained for each family with details of their Sickle Cell Status.

The Sickle Cell Status of every patient is entered in his medical file, which is maintained in the hospital. This is useful not only

to help correct diagnosis, but also to avoid unnecessary treatment.

A check-list to assess the clinical progress of patients with Homozygous Sickle Cell disease is maintained and the subject is assessed periodically.

The files and records are periodically updated, new records added, and, unwanted ones are deleted.

THE PATTERN OF INCIDENCE OF SICKLE CELL ANAEMIA IN THE SOLIGAS.

The statistical data obtained while screening the Soligas have been represented in the following Histograms and line graphs.

The main parameters considered are : Age, Sex, Homozygosity, Heterozygosity.

These graphs obtained with the help of the Computer, speak of themselves. The line graphs are placed below the Histograms for easy comparison. The line graphs enhance the variations observed in the Histograms. A total of 4 Histograms and 4 line graphs have been presented.

SUMMARY:

Data was present at the hospital, regarding Sickle Cell Status of nearly 2,000 tribals. Additional samples were collected from various tribal settlements and their Sickle Cell Status was found out as explained in the next chapter.

This data was tabulated and the percentage of each group, i.e., AS Females, AS Males, SS Females, SS Males were calculated to the total number of subjects (AA, AS, & SS) in each of the six age groups (0-5, 6-12, 13-18, 19-35, 36-50, More than 50).

With the above data, graphs were plotted with the help of a computer. With the help of these graphs, the following were concluded about Sickle Cell Anemia in the Soligas.

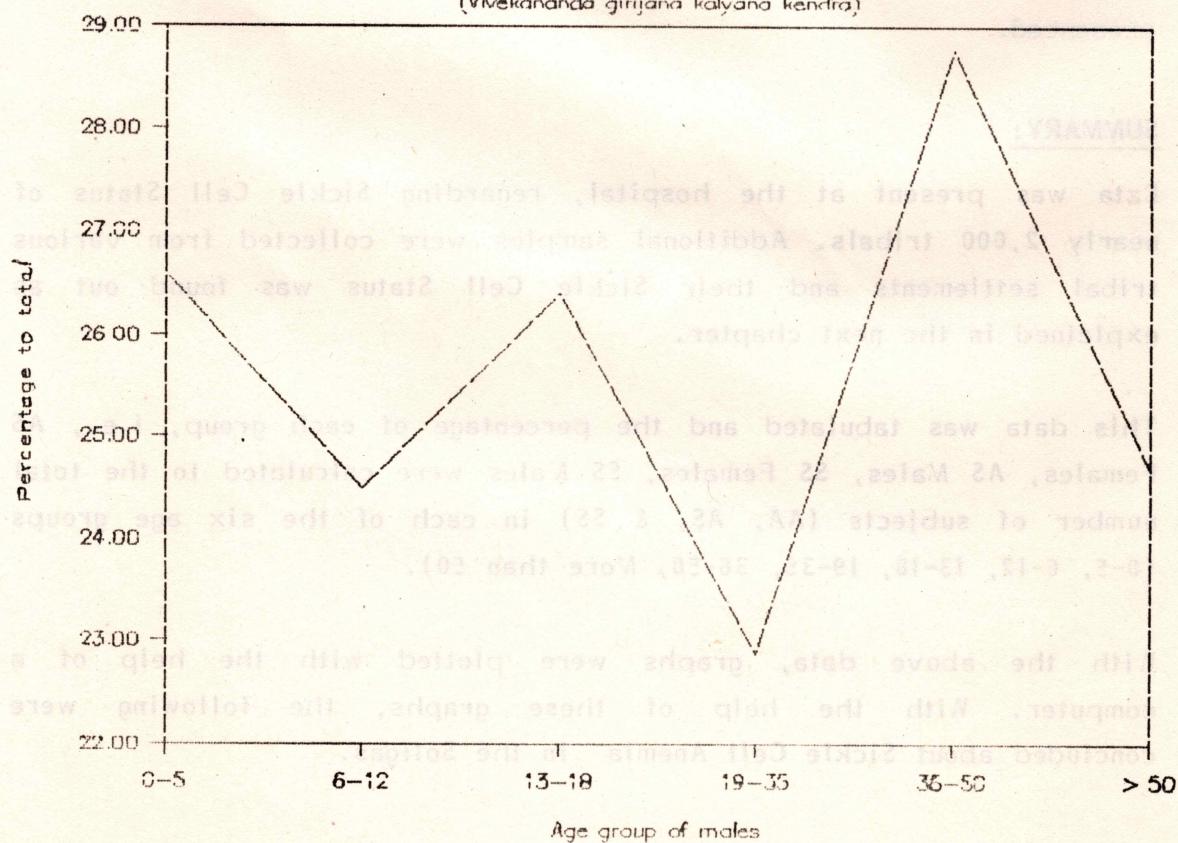
SICKLE CELL TRAIT (AS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



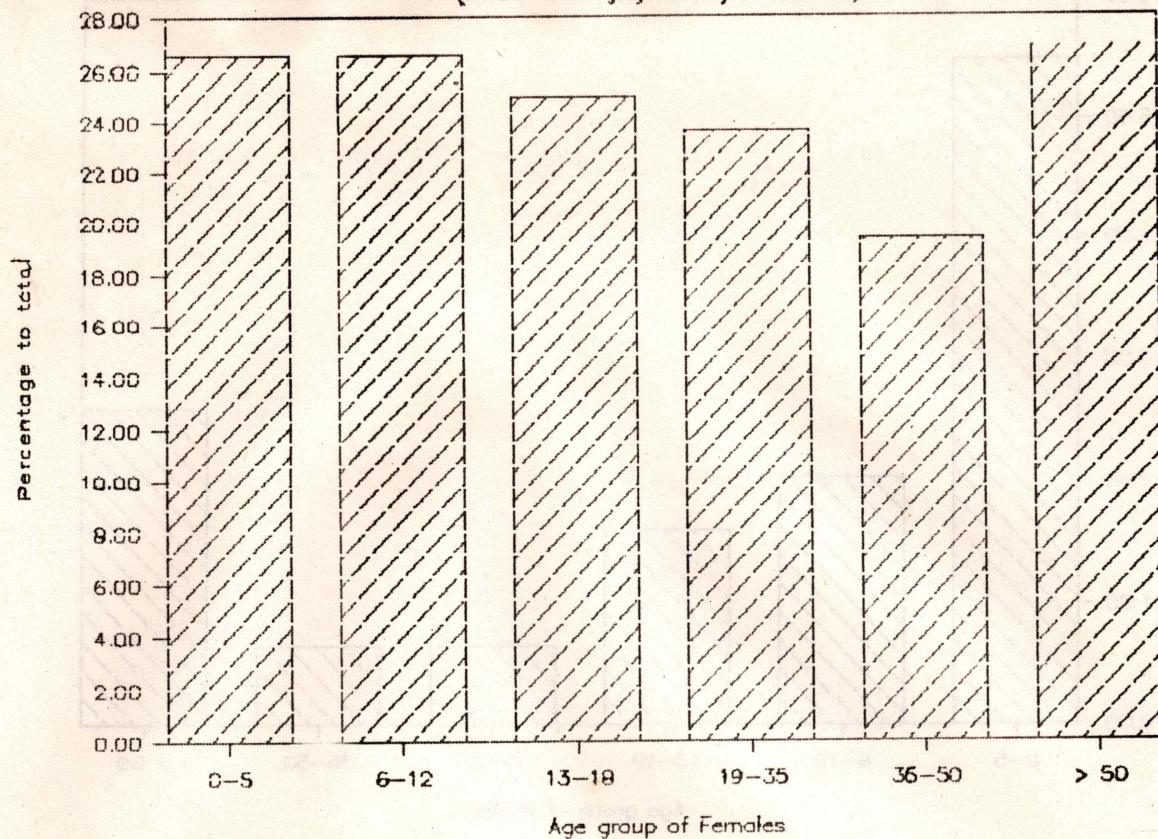
SICKLE CELL TRAIT (AS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



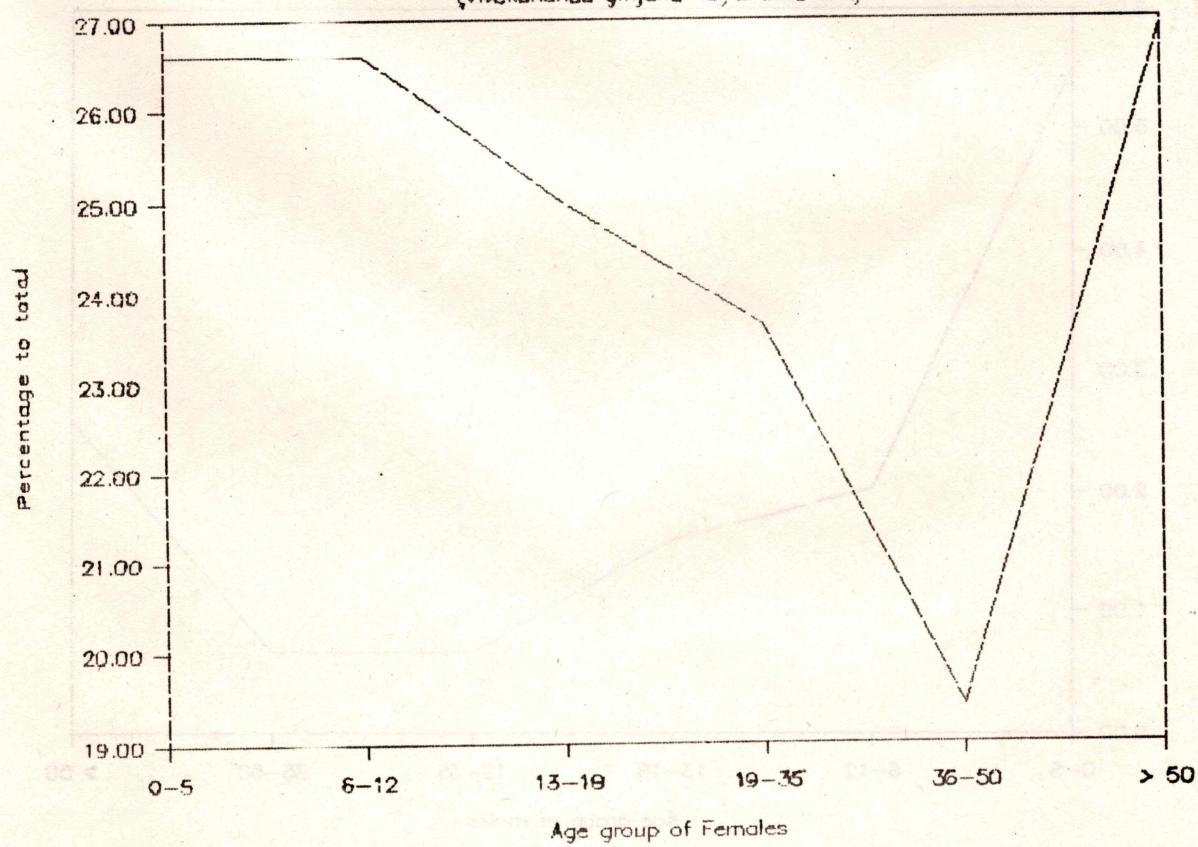
SICKLE CELL TRAIT (AS) IN THE SOLIGAS

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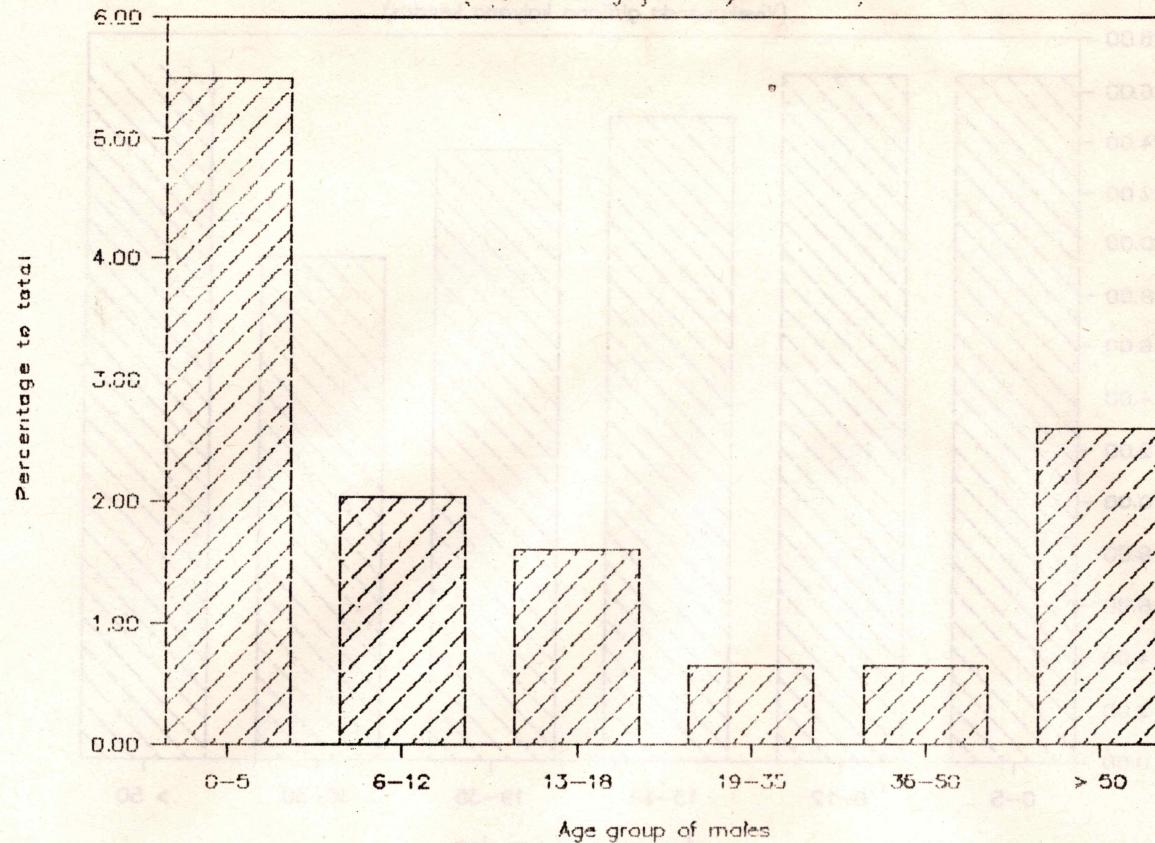
SICKLE CELL TRAIT (AS) IN THE SOLIGAS

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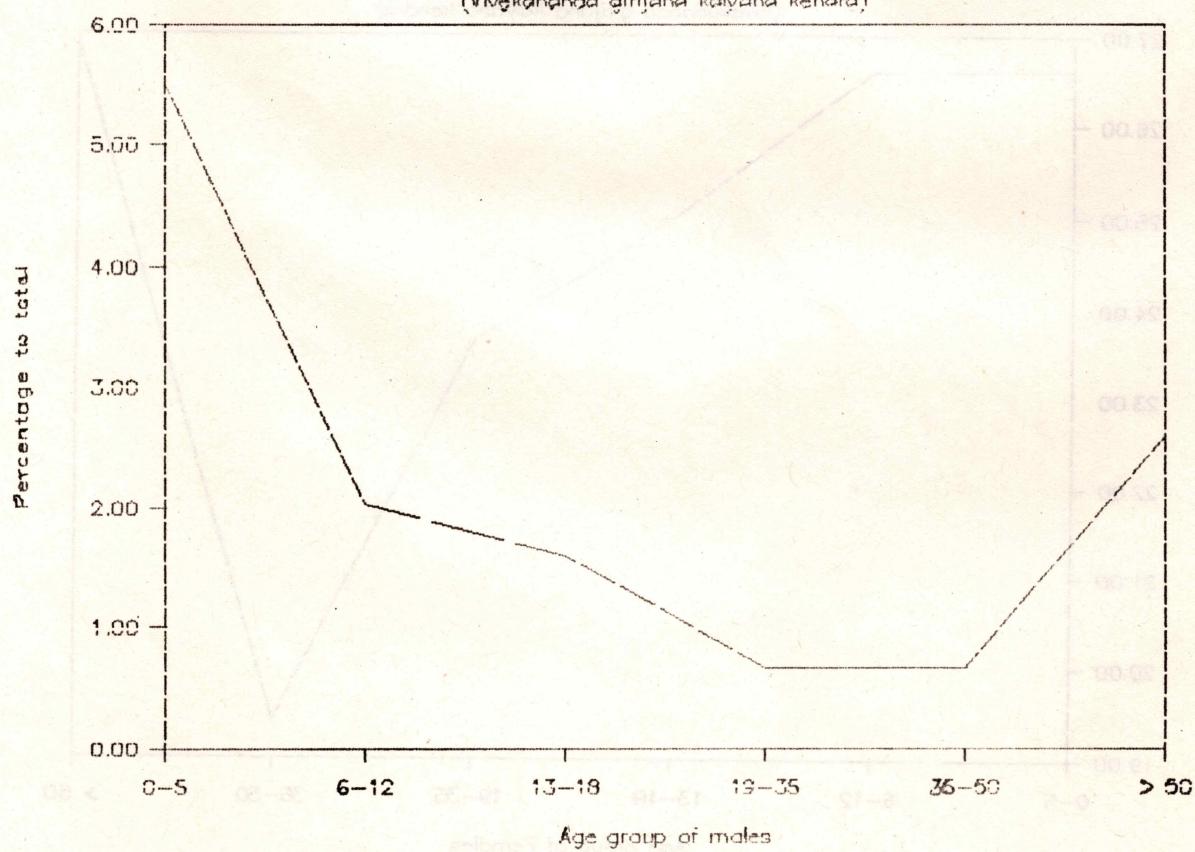
SICKLE CELL DISEASE (SS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



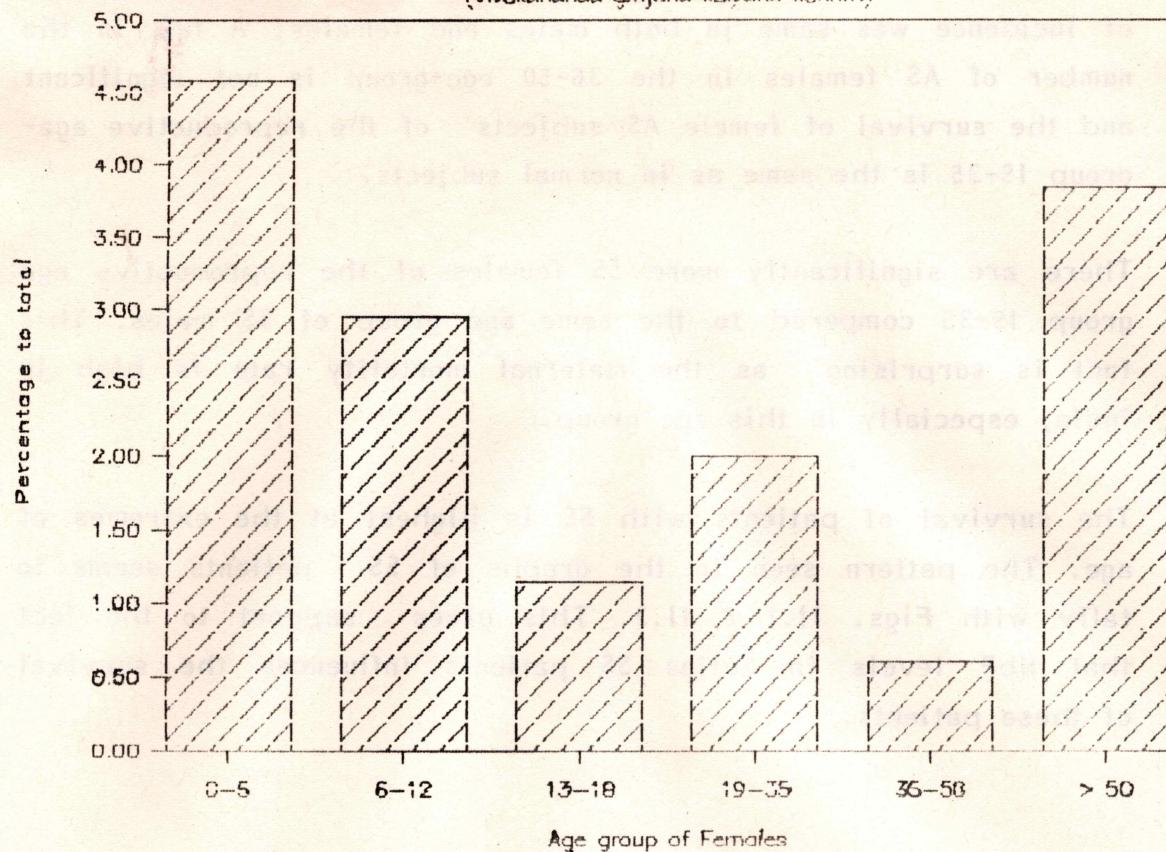
SICKLE CELL DISEASE (SS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



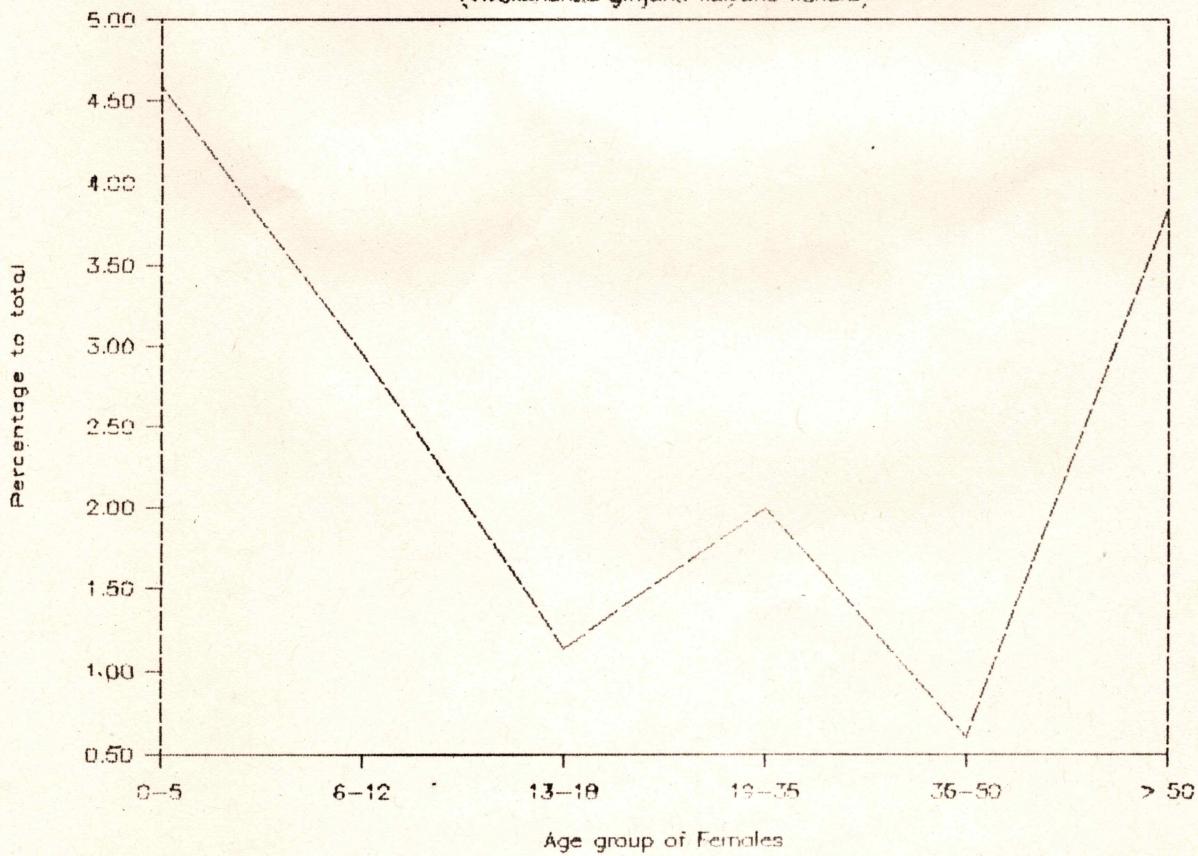
SICKLE CELL DISEASE (SS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



SICKLE CELL DISEASE (SS) IN THE SOLIGAS

(Vivekananda girijana kalyana kendra)



- * Sex did not influence the survival of AS subjects, as the pattern of incidence was same in both males and females. A fall in the number of AS females in the 36-50 age-group is not significant and the survival of female AS subjects of the reproductive age-group 19-35 is the same as in normal subjects.
- * There are significantly more SS females of the reproductive age group 19-35 compared to the same age group of SS males. This fact is surprising as the maternal mortality rate is high in India, especially in this age group.
- * The survival of patients with SS is highest at the extremes of age. The pattern seen in the graphs of SS patients seems to tally with Figs. II.1 & II.3. This gives support to the fact that HbF levels in Soliga SS patients influences the survival of these patients.

LABORATORY DIAGNOSIS

INTRODUCTION

" DONT TREAT THE TEST, NEITHER
THE SYMPTOM, NOT EVEN THE
DISEASE - TREAT THE PATIENT
AND THE PATIENT ALONE "

Laboratory diagnosis has a very important role in the detection of Sickle Cell Anaemia. The clinical manifestations are varied and there is no set clinical pattern in the course of the disease. Unless the physician is aware of the presence of the disease, he cannot diagnose sickle cell anaemia. There is every chance of a mis-diagnosis and its avoidable aftermath-wrong treatment, continued symptoms, waste of time, agony to both patient and doctor, waste of money, etc.

Another benefit of laboratory diagnosis is its role in genetic counselling. In populations where sickle cell anaemia is suspected, screening is done by drawing blood samples on a routine basis and subjecting it to various diagnostic tests. With these tests even the sickle cell trait can be identified. This helps in advising couples before they marry and in predicting the possibility of an offspring with sickle cell disease.

A special problem found by our field workers here is while collecting blood samples during screening of the population, the tribals are very hesitant to give their blood and a lot of time has to be spent convincing them a number of times and often being unsuccessful.

METHODS AVAILABLE

Many tests have been described to detect sickle cell anaemia.

A few of them are listed below :

- Testing of sickling of RBC's with a solution of sodium dithionite/sodium metabisulphite. (Sickling test).
- Testing for sickle cells with bacteria from the stools.
- Turbidity test.
- Filteration test.
- Electrophoresis :-

(i) With cellulose acetate

(ii) With starch gel

- Vertical

- Horizontal

Each test has its own set of advantages and disadvantages. Both the sickling test and the turbidity test can be used as 'Screening tests', but the turbidity test is preferred in mass screening.

One has to be very careful with the samples. Each sample is precious. Any mistake in handling the sample or in the testing method may destroy the sample. In one of our experiences, a sample was destroyed due to mild contamination of distilled water with acid !

METHODS USED IN OUR CENTRE

Let me repeat - EACH SAMPLE IS PRECIOUS !

I will briefly explain the methods followed in our laboratory. Let me first tell you about collecting the sample. A special disposable syringe and needle (Monovette) is used which contains EDTA. After drawing the blood sample, the plunger of the syringe can be broken and the blood sample can be stored in the syringe.

The blood samples are first subjected to a screening procedure by the turbidity test. This test detects the presence of sickle cell trait but it cannot differentiate between Homozygous (SS) and Heterozygous (AS) cases.

The test is as follows :

(i) Centrifuge the patients blood. Take the tube out of the centrifuge being careful not to disturb the deposit packed cells. With a Pasteur pipette remove some of the plasma so that there is an equal volume of plasma and packed cells in the tube. If the patient is not anaemic and has a normal packed volume, you will not need to remove any plasma.

But, if he is very anaemic you may have to remove quite a lot of plasma for there to be left an equal volume of plasma and packed cells. By doing this we are making sure that all specimens start this method with a more or less normal packed cell volume, and that anaemia is corrected for.

(ii) The working solution may be made as follows :

- (a) Weigh out 0.1g of sodium dithionite into a universal container. A small test tube marked at the 0.1g level will save you the need to weigh the sodium dithionite each time.
- (b) Add 10 ml of the buffer for haemoglobin solubility methods (13.5g potassium dihydrogen phosphate, 23.7g dipotassium hydrogen phosphate, 1g of white saponin, and upto 100 ml distilled water) to the container with the sodium dithionite in it. Mix the dithionite with the buffer until it is dissolved. This is the working solution and is enough for five tests. Be sure to make up this solution fresh each day. If there is any over at the end of the day, throw it away.

(iii) Pipette 1.9 ml of the working solution into a Kahn tube.

(iv) Add 0.2 ml of the blood.

(v) Put the Kahn tube containing the mixture of blood and working solution in front of the ordinary writing of a newspaper. Can you read the writing through the purple tube of solution ? If the solution is a clear purple and you can read through it, there is no haemoglobin S in the specimen, and the patients haemoglobin is AA.

If you cannot read a newspaper through the solution, because it is turbid, some haemoglobin S is present and the patient is either SS or AS.

Once we have a positive sample with the turbidity test, our job is to find out whether it is SS (Homozygous) or AS (Heterozygous). For this we have to do Electrophoresis. Cellulose Acetate Plates (Titan Plates) are used here for this purpose. A haemolysate (preparation of haemolysed cells) has to be prepared first. Here the plasma proteins are removed from the red cells by thorough washing with isotonic saline

and the cells are then lysed with water. The procedure is as follows:

- Take a sample of packed red cells (with plasma removed after centrifugation)
- Add equal volume of normal saline and centrifuge for five minutes. Remove supernatant and repeat the above steps 2-3 times.
- Add $\frac{1}{2}$ the volume of distilled water to the washed red blood cells - mix well. Keep for 5 minutes. This is the haemolysate.

In the next stage, a Tris-Glycine Buffer is prepared for the Electrophoresis chamber as follows :

Tris - 17.27g

Glycine - 2.63g

Distilled water upto 500 ml.

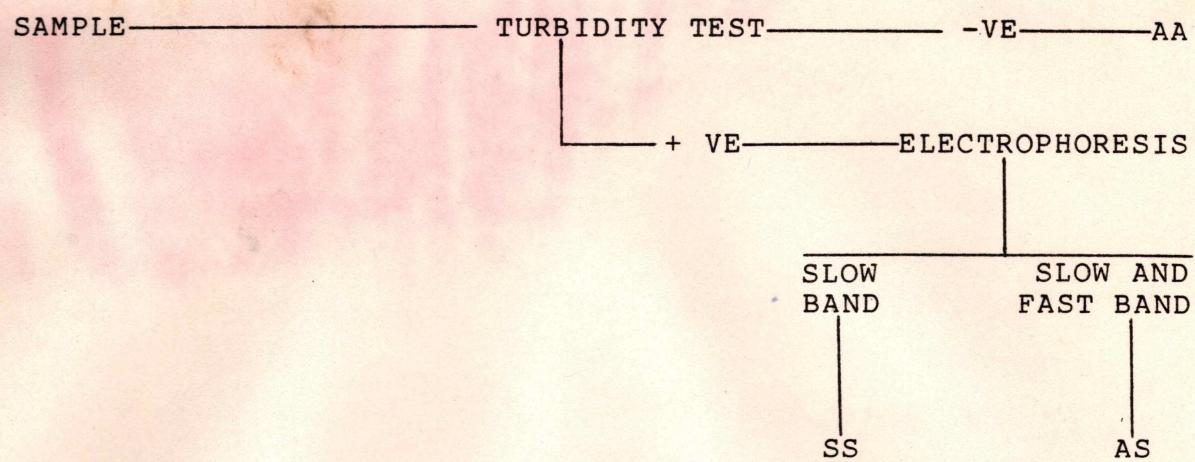
A reference line is drawn on the cellulose acetate plate and it is immersed slowly into the buffer without allowing any air bubbles and is kept for 15 minutes.

Now fill the electrophoresis chamber with the Tris - Glycine Buffer as indicated. Place the electrophoresis plate on the chamber in such a way that the reference line is towards the negative electrode.

Paint control samples of AA and SS with a sharp brush on the reference line and paint the unknown samples below them. (Note : The sample used is from the Haemolysate as described above)

Close the lid of the chamber and pass a current of 180 volts for atleast 20 minutes. The Electrophoretic mobility can be read now.

As mentioned earlier, there is net loss of two negative charges in the HbS molecule. So it moves slower towards the positive electrode as compared to HbA. There is a 'fast' HbA band and a 'slow' HbS band. Therefore, in Homozygous (SS) patients there is a single thick 'slow' band. In normal patients (AA) there is a single thick 'fast' band. In Heterozygous (AS) patients, there is both fast and slow bands.



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